Title
The Bb fragment of complement factor B acts as a B cell growth factor.

Journal
The Journal of experimental medicine, 168(4)

ISSN
0022-1007

Authors
Peters, MG
Ambrus, JL
Fauci, AS
et al.

Publication Date
1988-10-01

DOI
10.1084/jem.168.4.1225

Peer reviewed

THE Bb FRAGMENT OF COMPLEMENT FACTOR B ACTS AS A B CELL GROWTH FACTOR

By MARION G. PETERS, JULIAN L. AMBRUS, JR.,* ANTHONY S. FAUCI,* AND ERIC J. BROWN

*From the Departments of Medicine, Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri 63110; and the *Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892*

The production of antibodies after immunization requires antigen recognition by B cells, expansion of the pool of antigen-reactive B cells, and finally, differentiation of these B cells into antibody-secreting plasma cells. Current models for this process of B cell maturation in humans involve separate signals for B cell activation, proliferation, and differentiation (1, 2). One of the lymphokines that has been shown to enhance B cell proliferation is a 50–60-kD product of activated T cells and of the Namalwa cell line termed high molecular weight B cell growth factor (HMW-BCGF)[1] (3). Human HMW-BCGF has been purified to homogeneity, and this molecule has no obvious analog among the B cell growth factors of mice.

The environment of an ongoing immune response often includes the activated products of various inflammatory cascades, such as clotting or complement proteins. Multiple complement activation fragments have been shown to modulate the immune response, including C3a, C5a, C3b, C3d, and Ba (4–9). Engagement of B cell receptors for C3b (CR1) and C3d (CR2) by antibodies can also influence B cell proliferation (10, 11). The possibility that Bb can affect immune responses in vitro has been suggested (12) but has not been systematically investigated. In the present work, we have studied the modulatory influence of the complement activation fragment Bb on B lymphocyte function in detail. We have shown that Bb can induce activated but not unactivated B cells to proliferate and can act synergistically with IL-2 and B cell differentiation factors to induce B cell differentiation into antibody-secreting cells. This effect is mediated by direct interaction with B cells rather than by any effect of Bb on macrophage function (13). Interestingly, Bb has antigenic homology to HMW-BCGF, and its effects on B cell function are quite similar to those of HMW-BCGF. Moreover, Bb and HMW-BCGF compete for binding to the B cell plasma membrane. From these data we conclude that the Bb activation fragment of complement Factor B has a stimulatory effect on the proliferation of activated human B cells via a mechanism similar to HMW-BCGF, and that Bb may bind to the HMW-BCGF receptor on activated B cells. These data imply that at sites of inflammation, Bb may mimic the effects of this proliferative lymphokine.

This work was supported in part by National Institutes of Health grant GM-38330 (E. Brown). Address correspondence to Marion Peters, Washington University School of Medicine, Box 8124, 660 South Euclid Ave., St. Louis, MO 63110.

[1] *Abbreviations used in this paper:* CM, complete medium; HMW-BCGF, high molecular weight B cell growth factor; NPGB, paranitrophenyl paraquanidinobenzoate; SAC, *Staphylococcus aureus* Cowan I.

Thus, Bb may be a very early specific proliferative signal for B cells, to be followed after ingress and activation of T cells by the HMW-BCGF signal itself. This suggests a novel mechanism by which products of inflammatory reactions may influence the immune response.

Materials and Methods

Materials. Materials used were PHA (2 μg/ml; Wellcome Diagnostics, Beckenham, UK); IL-2 (2 IU/ml; Cellular Products Inc., Buffalo, NY); *Staphylococcus aureus* Cowan-I (SAC; 1:25,000, vol/vol; American Type Culture Collection, Rockville, MD); mixed lymphocyte culture supernatants; and polyclonal anti-Factor B antibody (Cytotech, San Diego, CA). HMW-BCGF was the purified product of PHA-stimulated Namalwa cells (3). Mixed lymphocyte culture supernatant has been shown to contain both IL-2 and B cell differentiation factor activity (14). BCGF-1C2 is an mAb that recognizes HMW-BCGF (3). P3X is a mouse IgG1 control mAb with no known specificity.

Complement Component Purification. C3, C3b, Factor B, and Factor D were purified as previously described (15–18). For some Western blots, commercially available Factor B was used (Cytotech). Ba and Bb were prepared by incubating C3b at 1.5 mg/ml with Factor B (10 mg/ml) and Factor D (10 μg/ml) for 1 h at 37°C (19). The Bb and Ba were repurified by DEAE-Sephacel chromatography (13) followed by chromatography on ACA-54 and anti-C3-Sepharose. In some cases the Ba and Bb each were further purified by TSK-3,000 gel permeation chromatography. Under these conditions the Bb and the Ba exhibit single homogenous peaks by SDS-PAGE. For some experiments the Bb was treated with an irreversible serine protease inhibitor, paranitrophenyl paraguanidinobenzoate (NPGB), for 30 min at room temperature. Excess NPGB was removed by extensive dialysis with saline before use. Bb so treated lost enzymatic activity as assessed by hydrolysis of *N*-acetyl-glycl-lysyl-methyl ester (20), assayed as described (21).

Cell Purification. Human mononuclear cells were purified from heparinzed peripheral blood by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) gradient centrifugation (22). Human tonsils and spleens were purified by dispersing tissue through mesh into single cell suspensions and mononuclear cells obtained by Ficoll-Hypaque. B and T cells were obtained from mononuclear cells by monocyte depletion (glass bead adherence and G-10 columns) followed by rosetting with 2-aminoethylthiouronium bromide–treated sheep RBC (23–25). B cells were 80–95% surface Ig positive and <2% "E" rosette positive. T cells were >95% "E" rosette positive. In all assays, the incorporation of [^{3}H]TdR in response to PHA was assessed to ensure the functional purity of the B cells. In some experiments, B cells were further separated by size into small, medium, and large populations using counter-current elutriation as described (26). Monocytes were obtained by adherence or counter-current elutriation (26). The MB cell line is an EBV-transformed cell line from a normal individual, cultured and maintained as described (27).

Cell Proliferation. 10^5 cells were cultured in 0.2 ml complete medium (CM; 10% heat-inactivated FCS in RPMI 1640 with 50 μg/ml gentamicin) in the presence of SAC and various cytokines. Proliferation was measured by [^{3}H]TdR incorporation over the last 18 h of a 96-h culture. In some experiments, 10^5 monocytes were cultured in 1 ml CM for 4 and 24 h with and without Bb (10 μg/ml; 170 nM) and the supernatants were obtained. 10^4 monocytes were also incubated overnight with Bb at 10 μg/ml (170 nM). The monocytes were then washed and placed in culture with 10^5 SAC-activated B cells in 0.2 ml CM.

Western Blot Analysis. Analysis was performed as described (3), using transfer to Immobilon paper (Millipore Continental Water Systems, Bedford, MA). SDS-PAGE was performed without reduction on 10% acrylamide gels.

Ligand Binding Experiments. Bb was labeled with Na ^{125}I using chloroglycoluril (28). Various preparations had specific activities ranging from 2.5 to 5.3 μCi/nmol. For binding curves and Scatchard plots, 10^6 B cells, or MB cells, with or without 6 μM (360 μg/ml) unlabeled ligand, were incubated with various concentrations of ^{125}I-Bb at 4°C for 2 h. Bound and free ligand were separated by centrifugation of the reaction mixture through 250 μl Versi-

lube F-50 (General Electric Co., New York, NY) at 12,500 *g*, following which, the cell pellet (bound ligand) and the supernatant (free ligand) were counted separately in a gamma counter. Specific binding was calculated as binding in tubes without excess cold Bb minus binding in tubes containing excess cold Bb. Under these conditions nonspecific binding represented 0.22 ± 0.01% of input cpm. All determinations were performed in duplicate.

Results

Effect of Bb on B Cell Function. Because of the possibility that Bb could affect immune responses (12), we systemtically examined the effect of Factor B and its larger cleavage fragment, Bb, on cellular immune function. Preliminary data suggested that Bb did not influence T cell proliferation in response to PHA. Bb also did not influence activation of purified resting B cells. In the absence of activation of the B cells by SAC, Bb had little effect on subsequent proliferation (Fig. 1). However, after the B cells had been activated with SAC, Bb markedly enhanced proliferation in a dose-dependent manner (Figs. 1 and 2 *A*). On the other hand, intact Factor B had no effect on the proliferation of unactivated or activated B cells (data not shown). Bb also enhanced B cell proliferation in the presence of suboptimal HMW-BCGF concentrations in a dose-dependent manner (Fig. 2 *B*). However, maximal proliferation in the presence of HMW-BCGF and Bb was no greater than in the presence of Bb alone (compare Fig. 2 *A* and *B*) or in the presence of optimal HMW-BCGF alone (data not shown), suggesting that the effects of HMW-BCGF and Bb were additive, and not synergistic. Since Bb enhanced only the proliferation of B cells that had been activated in vitro by SAC, we tested the effect of Bb on proliferation of in vivo preactivated B cells. These cells, which presumably have been stimulated by antigen in vivo, are large and can be isolated from smaller unstimulated B cells by counter-current elutriation (26). As shown in Fig. 3, Bb markedly enhanced proliferation of these larger activated B cells at concentrations >1 μg/ml (~17 nM), while its effect on smaller B cells was far less marked. Thus, the larger preactivated B cells are more susceptible to the effect of Bb. These data suggested a functional similarity between Bb and HMW-BCGF. Like Bb, HMW-BCGF enhances proliferation of SAC-stimulated, or in vivo preactivated B cells, but has little effect on small unactivated B cells (29). In addition, Bb had no effect on Ig production when added alone or with SAC, which is also similar to HMW-BCGF (29). This, and the fact that Bb could not further enhance proliferation of B cells optimally stimulated with HMW-

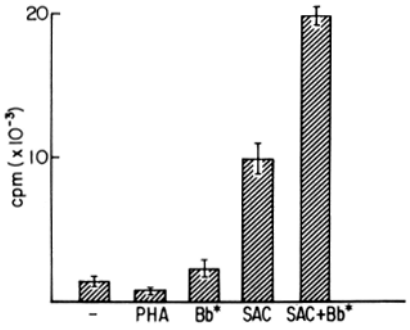

FIGURE 1. Bb enhanced the proliferation of SAC-stimulated B cells but had no significant effect on its own. [^{3}H]T incorporation by B cells activated by SAC, PHA (2 μg/ml), and Bb (1 μg/ml). Mean ± SD of three experiments.

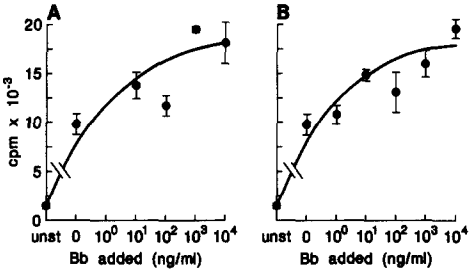

FIGURE 2. Bb enhanced the proliferation of SAC-stimulated B cells alone (*A*) and in the presence of suboptimal concentrations of HMW-BCGF. (*A*) Mean ± SD of [^{3}H]T incorporation by unstimulated B cells (*unst*) and B cells stimulated with SAC and Bb (1–10,000 ng/ml). (*B*) Mean ± SD of [^{3}H]T incorporation by unstimulated and B cells stimulated with SAC, 10% HMW-BCGF, and varying concentrations of Bb. The proliferative effect of Bb was dose responsive over this range.

BCGF suggested that the two proliferative stimuli might be acting through a similar mechanism.

This hypothesis suggested that the effect of Bb was directly on B cells rather than on monocytes, a previously described target of Bb action (13). To test whether Bb acted indirectly on B cell proliferation via modulation of monocyte function, we performed two experiments. In one, monocytes were cultured for 4 and 24 h in the presence or absence of 10 μg/ml (170 nM) of Bb. Supernatants were harvested and then placed in culture with SAC-activated B cells. Monocyte supernatants with or without Bb stimulated SAC-activated B cell proliferation; thus, the effect of Bb could not be evaluated under these conditions. Therefore, we performed a second series of experiments in which monocytes were cultured overnight in the presence of Bb at 10 μg/ml (170 nM). After this preincubation the cells were washed and placed in culture with SAC-activated B cells. Preincubation of monocytes with and without Bb had similar effects on subsequent B cell [^{3}H]Tdr incorporation (1,884 ± 227 and 1,331 ± 169 cpm, respectively), which was above unstimulated cultures (293 ± 65) but less than optimally stimulated cultures (26,876 ± 1,633 cpm). Thus, Bb did not enhance monocytes' ability to affect B cell proliferation.

Next, we evaluated the effect of the serine protease inhibitor NPGB on B cell proliferation induced by Bb. Because Bb's effect on macrophage spreading requires preservation of its serum esterase activity (21), we examined whether this was true for its enhancement of B cell proliferation. If the proteolytic activity of Bb were required, it might also be true that HMW-BCGF enhances proliferation via a protease ac-

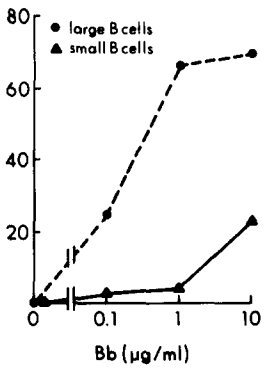

FIGURE 3. Bb enhanced the proliferation of larger in vivo activated B cells (●) more than smaller resting B cells (▲). B cells were separated by size and stimulated with SAC and 10% HMW-BCGF. Bb was added as indicated. The ordinate shows the mean percent increase in [^{3}H]T incorporation of triplicate samples compared with the absence of Bb.

TABLE I

Serine Protease Inhibitor NPGB Does not Inhibit Bb or HMW-BCGF-stimulated Proliferation of SAC-activated B Cells

SAC plus:	[^{3}H]TdR incorporation	
	Buffer*	NPGB*
	cpm	
-	22,989 ± 3,931‡	-
Bb (1 μg/ml)	56,226 ± 4,366	50,888 ± 3,145
10% BCGF	48,204 ± 1,950	50,501 ± 2,101
10% BCGF + Bb (1 μg/ml)	79,526 ± 6,936	66,350 ± 10,352

* Bb was treated with NPGB or buffer, dialyzed, and then added to SAC-stimulated B cells.

‡ Mean ± SD of triplicate determinations.

tivity. However, as shown in Table I, neither the proliferative effect of Bb nor of HMW-BCGF was diminished by treatment with NPGB. Thus, the mechanism of enhancement of B cell proliferation by Bb is quite distinct from its effects on macrophage spreading. These data present additional evidence that the effect of Bb on B cell proliferation is not mediated via an indirect activation of a few contaminating macrophages in the B cell preparations.

Bb Binds to B Lymphocytes. The hypothesis that Bb acts directly on B cells to effect proliferation suggests that there is a binding site for Bb on B cells. We examined the binding of ^{125}I-Bb to SAC-stimulated tonsillar B cells and to the MB cell line. ^{125}I-Bb bound saturably to preparations of activated tonsillar B cells, as demonstrated by the ability of unlabeled ligand to inhibit the binding of the radiolabel (Fig. 4 *A*). Remarkably, HMW-BCGF also inhibited the binding of ^{125}I-Bb to activated B cells, suggesting that Bb and HMW-BCGF bound to the same receptor on these cells. The unrelated molecules IgG and Ba did not affect the binding of ^{125}I-Bb. To ensure that this binding was to B cells rather than to contaminating macrophages, we also examined the binding of ^{125}I-Bb to the EBV-transformed B cell line, MB (Fig. 4 *B*). MB exhibits many of the surface characteristics of activated B cells, including expression of the receptor for HMW-BCGF (27). MB also bound Bb specifically, and as with activated untransformed B cells, this binding was inhibited

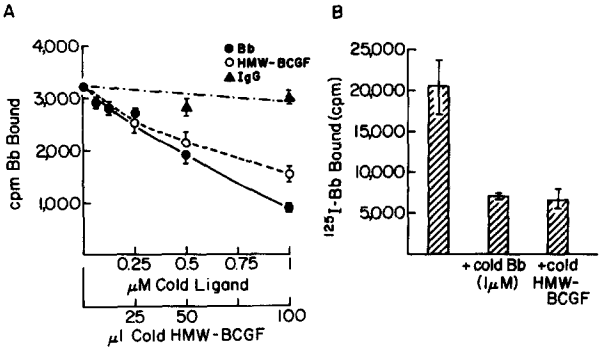

FIGURE 4. Bb binding to B cells and an EBV-transformed B cell line. ^{125}I-Bb at 30 μg/ml (500 nM) was incubated with 10^6 SAC-activated tonsillar B lymphocytes (*A*) or with 2 × 10^6 MB cells (*B*) as described in Materials and Methods. Both unlabeled Bb and unlabeled HMW-BCGF inhibited binding of ^{125}I-Bb to these cells. Neither IgG (▲) nor Ba (data not shown) inhibited ^{125}I-Bb binding.

by cold HMW-BCGF. Scatchard analysis of the saturable binding of Bb to SAC-activated normal B cells and MB cells was performed. As shown in Fig. 5, Bb bound to activated normal B cells with a Kd of 110 ± 19 nM (6.6 μg/ml) and there were 21,000 ± 3,500 sites per cell (mean ± SEM, n = 3). Analysis of binding to MB cells showed a sightly lower affinity (Kd: 152 ± 19 nM: 9 μg/ml) with more binding sites per cell (54,000 ± 8,000, mean ± SEM, n = 4). Together these data demonstrate that there is a Bb receptor on the activated B cells themselves, and that the Bb receptor is closely related or identical to the B cell receptor for HMW-BCGF.

Factor B and Bb Are Antigenically Related to HMW-BCGF. Because of the similarity in functional effect of Bb and HMW-BCGF, as well as their similar molecular weights and apparent crossinhibition of binding to B cells, we examined the two molecules for antigenic homology. We first probed with BCGF-1C2, an mAb to HMW-BCGF, which inhibits its function (3). As shown in Fig. 6, BCGF-1C2 bound to both Bb (lane *3*) and Factor B (lane *5*), as well as HMW-BCGF (lane *1*). BCGF-1C2 did not recognize Ba (lane *4*) or proteins in 10% FCS (lane *2*). BCGF-1C2 bound to both Factor B made in our laboratory and to commercially prepared Factor B, both of which showed a single band on SDS-PAGE. The binding to Factor B was quite specific, since it was the only protein recognized by BCGF-1C2 when a 12–30% PEG fraction of normal human plasma was subjected to electrophoresis and then analyzed by Western blot analysis (data not shown). Using a polyclonal anti-Factor B antibody, we performed reciprocal experiments to look for binding of this antibody to HMW-BCGF. As shown in Fig. 7, the polyclonal anti-Factor B recognized HMW-BCGF (lanes *1* and *2*) as well as Bb (lane *3*), and Ba (lane *4*). The Coomassie stains of the FB preparations used are shown in Fig. 7 *B* with Ba in lane *1*; Bb in lane *2*; and FG in lane *3*. These experiments with antibodies to both Factor B and HMW-BCGF demonstrated antigenic homology between Factor B and HMW-BCGF. Of particular interest was the finding that BDGF-1C2, which inhibits the function of HMW-BCGF, crossreacted with Bb. This suggested that the close functional homology between the two molecules might be mediated via structurally similar domains that reacted with the B cell plasma membrane.

Monoclonal anti-HMW-BCGF Inhibits the Proliferative Signal of Bb. The data obtained in the preceding studies suggested that Bb and HMW-BCGF might be homologous in a domain recognized by a B cell receptor important in signalling cellular proliferation. To test this directly, we used the mAb BCGF-1C2, which inhibits HMW-BCGF action on B cells (3), and which binds to Bb (see Fig. 6). As shown in Table II, BCGF-

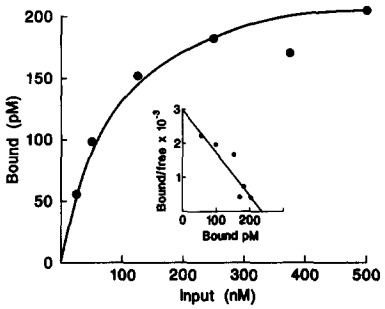

FIGURE 5. The specific binding of ^{125}I-labeled Bb to SAC-stimulated B cells was measured and plotted against the amount of ^{125}I-labeled Bb added (*input*). The inset shows Scatchard plot of this Bb binding to SAC-stimulated B cells.

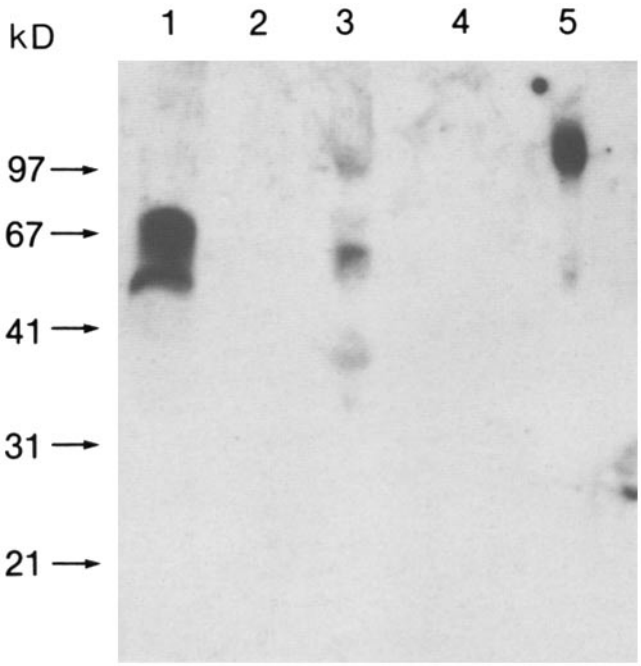

FIGURE 6. Antibody to HMW-BCGF recognized Bb. BCGF-IC2, an mAb to HMW-BCGF was used in a Western blot analysis of HMW-BCGF (lane *1*); 10% FCS (lane *2*); Bb (lane *3*); Ba (lane *4*); and Factor B (lane *5*). BCGF-IC2 recognized Factor B and Bb, but not Ba by this procedure. The following amounts of protein were loaded on the gel: HMW-BCGF, 0.6 μg (10 pm); Bb, 3.6 μg (60 pm); and Ba, 2.95 μg (84 pm).

1C2 inhibited Bb-induced proliferation of SAC-activated B cells in a dose-dependent manner. BCGF-1C2 also inhibited proliferation induced by HMW-BCGF. P3X, a control antibody, had no effect on Bb-induced proliferation when used at similar concentrations. Thus, BCGF-1C2 recognized epitopes on both Bb and HMW-BCGF, which are in proximity to sites required for their proliferative activity.

Because of the close relationship between Bb and HMW-BCGF in their effects on B cell function, we sought to determine whether HMW-BCGF could affect complement activation. However, the limited availability of this protein precluded direct experiments. BCGF-1C2, which inhibits HMW-BCGF and Bb activity on B cells, did not affect C3b and Factor D–dependent Factor B cleavage or Bb-mediated C5 cleavage (data not shown).

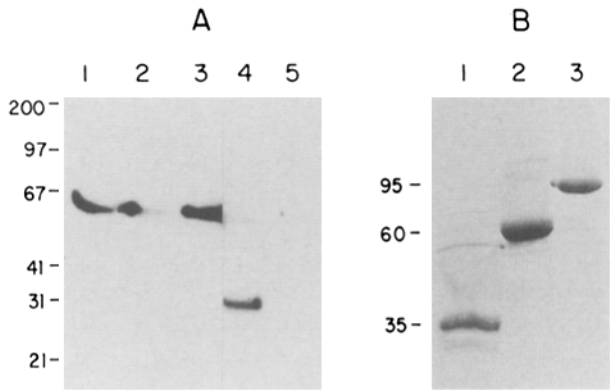

FIGURE 7. (*A*) Polyclonal antiserum to Factor B recognized HMW-BCGF. A polyclonal antiserum to Factor B was used in a Western blot analysis; lane *1*, HMW-BCGF containing supernatant; lane *2*, purified HMW-BCGF; lane *3*, Bb; lane *4*, Ba; and lane *5*, 5% human serum albumin. Identical amounts of protein were loaded as in Fig. 6. (*B*) Coomassie blue stain of Ba (lane *1*), Bb (lane *2*), and FB (lane *3*) used in the functional and binding experiments described in this paper.

TABLE II

Bb-induced B Cell Proliferation Is Inhibited by Antibody to HMW-BCGF

SAC plus:	[^{3}H]TdR incorporation
	cpm
Bb, 10 μg/ml	16,221 ± 2,311*
Bb + BCGF-1C2, 0.2 μg/ml	23,584 ± 1,567
1.0 μg/ml	2,758 ± 43
Bb + P3X, 1.0 μg/ml	24,678 ± 1,190
HMW-BCGF 25%	22,352 ± 516
BCGF + BCGF-1C2, 0.2 μg/ml	13,968 ± 2,449
1.0 μg/ml	514 ± 22
-	2,693 ± 387
BCGF-1C2, 1 μg/ml	1,216 ± 13

B cells were stimulated with SAC for 48 h, washed, and then added in culture as indicated.

* Mean ± SD of triplicate experiments.

Discussion

In this work we have examined the relationship between Bb, the 60-kD protease-containing activation fragment of complement Factor B, and HMW-BCGF, a well-described B cell growth factor (3). There is both antigenic and functional homology between these molecules. First, antibodies made against Factor B recognize HMW-BCGF and, conversely, antibodies made to HMW-BCGF recognize both intact Factor B and Bb. This is particularly significant in the case of the mAb BCFG-1C2, which inhibits HMW-BCGF binding to B cells (3). Because this antibody recognizes an epitope on BCGF-1C2 at or near the cell-binding site of the molecule, we examined whether the crossreactive antigen, Factor B, might affect B cell proliferation. We found no effect of intact Factor B, but did find a significant enhancing effect of Bb on B cell proliferation. The effect of Bb shared many characteristics with the known functions of HMW-BCGF. Neither would stimulate unactivated B cells to proliferate, yet both could enhance proliferation of SAC- or anti-IgM-activated B cells. Both exerted their effect primarily on large in vivo preactivated B cells. Both had no effect on Ig secretion when added alone. These data suggest that Bb and HMW-BCGF have functional, as well as antigenic, homology. Because Bb is serine protease, we tested whether its proteolytic activity was required for its effect on B cell proliferation. NPGB, an irreversible serine protease inhibitor, had no effect on the ability of either Bb or HMW-BCGF to induce B cell proliferation. This demonstrated that the effect of Bb on B cells was quite distinct from its effect on monocyte spreading, which clearly is dependent on the protease activity of Bb (21). We do not believe that Bb acts indirectly on B cell proliferation via stimulation of monocytes because (*a*) it binds directly to SAC-stimulated B cells and an EBV-transformed B cell line; (*b*) preincubation of monocytes with Bb did not lead to enhanced B cell proliferation; and (*c*) the activity of Bb on B cells was not inhibited by NPGB.

Because proteolytically inactive Bb affected B cell proliferation, we hypothesized that its effect might be receptor mediated. Bb bound in a saturable manner to B cells, which was consistent with a receptor-mediated event. Although the Kd for Bb binding was rather high, the half maximal biological effect of Bb was in a potentially physiologic range, perhaps suggesting that only a small percent receptor oc-

cupancy was sufficient to transmit Bb's proliferative signal. Even more striking, HMW-BCGF inhibited the binding of Bb to B cells, suggesting that the two molecules bound to identical or closely related sites on the plasma membrane. Taken together these data demonstrate that Bb and HMW-BCGF have antigenic homology, including a site known to be involved in HMW-BCGF binding to its receptor, and that Bb and HMW-BCGF have functionally similar effects on B cell proliferation. Thus, the ability of Bb and HMW-BCGF to compete with each other for membrane binding strongly suggests that these two molecules share a receptor expressed on activated B cells and on the MB line.

These data raise the possibility that HMW-BCGF is, in fact, Bb. We believe that this is not the case for the following reasons. First, HMW-BCGF is synthesized by T cells and some B cell lines, while Factor B is synthesized by monocytes. There is no evidence for its synthesis by lymphocytes of any type. Second, pulse-chase experiments have not suggested a larger molecular weight precursor for HMW-BCGF as would be expected if it were derived from Factor B (12). Since BCGF-1C2 recognized intact Factor B, this precursor would have been found if it were present. Finally, although precise quantitation of the HMW-BCGF protein is difficult, estimates of its potency suggest that it induces B cell proliferation at concentrations at least 1,000-fold less than the concentrations of Bb required to induce B cell proliferation. Thus, although there is homology in the cell-binding epitopes of Bb and HMW-BCGF, these are clearly distinct molecules.

The data presented here lead us to hypothesize a new mechanism by which peptides produced at sites of inflammation or infection can affect immune function. Since HMW-BCGF alone does not induce differentiation of B cells into plasma cells, its primary role in vivo is likely to be to provide a signal for the clonal expansion of antigen-stimulated B cells. Our work suggests that Bb can have a physiologic role similar or identical to HMW-BCGF. Assuming that the concentration of Factor B present at a site of inflammation is approximately equal to serum (2.5 μM), cleavage of only 1–5% of Factor B would achieve concentrations of Bb capable of significant stimulation of the growth of antigen-activated B cells. Thus, Bb could be a signal for the proliferation of antigen-activated B cells, which is generated early in the inflammatory process. HMW-BCGF, which is synthesized by activated T cells, would not be present until later times after the inflammatory event, and could serve to sustain the clonal expansion of antigen-activated B cells. Other complement components, lymphokines, and inflammatory molecules will also act to regulate B cell function at sites of inflammation. The net effect on B cell physiology would be the sum of a very complex set of stimuli. Nonetheless, this receptor-mediated, nonprotease effect of Bb suggests a mechanism through which complement activation, as part of a nonspecific response to antigenic challenge, can provide an early signal to enhance the antigen-specific immune response.

Summary

The process of B cell growth and differentiation into plasma cells is highly regulated and may be influenced by a large number of inflammatory mediators, including complement components. We have studied the regulatory influence of Bb, a 60-kD peptide created during the cleavage of complement Factor B by Factor D and C3b. Purified Bb alone had no effect on proliferation and differentiation of human splenic

or tonsillar B cells. However, when B cells were activated by *Staphylococcus aureus* Cowan I (SAC), Bb enhanced proliferation in a dose-dependent manner. Bb also enhanced proliferation when cocultured with SAC and suboptimal concentrations of purified 60-kD B cell growth factor (HMW-BCGF), a previously described lymphokine that is known to possess growth-promoting activity. However, Bb had no effect on cells treated with optimal concentrations of HMW-BCGF. Like HMW-BCGF, Bb's major effect was on the larger in vivo activated B cells. Half-maximal enhancement of proliferation was reached at a Bb concentration of 1–10 nM. Of note is the fact that antibody to Factor B recognized HMW-BCGF, and an mAb to HMW-BCGF also recognized Factor B and Bb, but not Ba. Moreover, radiolabeled Bb bound saturably to activated B cells and to an EBV-transformed human B cell line. The binding of Bb was inhibited by HMW-BCGF but not by Ba or IgG. Thus, Bb is antigenically and functionally related to HMW-BCGF, and can act as a B cell growth and differentiation factor at potentially physiologic concentrations. These data suggest that Bb may be important in amplifying the immune response in areas of inflammation. Since complement activation occurs at inflammatory sites long before induction of HMW-BCGF synthesis, Bb may be an early signal for the clonal expansion of antigen-activated B cells.

The authors thank Ms. Joyce Rudolph for excellent secretarial assistance and Patrick McFarland, Howard Mostowski, Norma Witzel, and Alex Zheleznyak for technical assisitance.

Received for publication 30 July 1987 and in revised form 20 June 1988.

References

1. Kehrl, J. H., A. Muraguchi, J. L. Butler, R. J. M. Falkoff, and A. S. Fauci. 1984. Human B cell activation, proliferation and differentiation. *Immunol. Rev.* 78:75.
2. Howard, M., K. Nakanishi, and W. E. Paul. 1984. B cell growth and differentiation factors. *Immunol. Rev.* 78:185.
3. Ambrus, J. L., C. H. Jurgensen, E. J. Brown, and A. S. Fauci. 1985. Purification to homogeneity of a high molecular weight human B cell growth factor. *J. Exp. Med.* 162:1319.
4. Morgan, E. L., W. O. Weigle, and T. E. Hugli. 1982. Anaphylatoxin-mediated regulation of the immune response. *J. Exp. Med.* 155:1412.
5. Berger, M., and T. A. Fleischer. 1983. Native C3 does not bind to the C3b receptor (CR1) of human blood B lymphocytes or alter immunoglobulin synthesis. *J. Immunol.* 130:1021.
6. Mueth, J. L., E. L. Morgan, R. G. DiScipio, and T. E. Hugli. 1983. Suppression of T lymphocyte functions by human C3 fragments. *J. Immunol.* 130:2605.
7. Weiler, J. M., Z. K. Ballas, T. L. Feldbush, and B. W. Needleman. 1982. Inhibition of human lymphocyte blastogenesis by C3: the role of serum in the tissue culture medium. *Immunology.* 45:247.
8. Gooman, M. G., D. E. Chenoweth, and W. O. Weigle. 1982. Potentiation of the primary humoral immune response in vitro by C5a anaphylatoxin. *J. Immunol.* 129:70.
9. Praz, P., and E. Ruuth. 1986. Growth-supporting activity of fragment Ba of the human alternative complement pathway for activated murine B lymphocytes. *J. Exp. Med.* 163:139.
10. Daha, M. R., A. C. Bloem, and R. E. Bullieux. 1984. Immunoglobulin production by human peripheral lymphocytes induced by anti C3 receptor antibodies. *J. Immunol.* 132:1197.
11. Melchers, F., A. Erdei, T. Schulz, and M. P. Dierich. 1985. Growth control of activated synchronized murine B cells by the C3d fragment of human complement. *Nature (Lond.).* 317:264.

12. Hirani, S., D. S. Fair, R. A. Papain, and J. S. Sundsmo. 1985. Leukocyte complement: Interleukin-like properties of Factor Bb. *Cell. Immunol.* 92:235.
13. Gotze, O., C. Bianco, and Z. A. Cohn. 1979. The induction of macrophage spreading by Factor B of the properdin system. *J. Exp. Med.* 149:372.
14. Peters, M., J. L. Butler, J. B. Margolick, and T. L. Gerrard. 1985. Synergy of helper factors in the differentiation of in vivo preactivated antigen specific human B cells. *Cell. Immunol.* 91:31.
15. Voegel, C. W., and H. J. Muller-Eberhard. 1984. Cobra venom factor: improved method for purification and biochemical characterization. *J. Immunol. Methods.* 73:203.
16. Hammer, C. H., G. H. Wirtz, L. Renfer, H. D. Gresham, and B. F. Tack. 1981. Large scale isolation of functionally active components of the human complement system. *J. Biol. Chem.* 256:3995.
17. Lambris, J. D., N. J. Dobson, and G. D. Ross. 1980. Release of endogenous C3b inactivator from lymphocytes in response to triggering membrane receptors for βIH globulin. *J. Exp. Med.* 152:1625.
18. Lesavre, P. H., T. E. Hugh, A. F. Esser, and H. J. Muller-Eberhard. 1979. The alternative pathway C3/C5 convertase: chemical basis of Factor B activation. *J. Immunol.* 123:529.
19. Christie, D. L., J. Gagnon, and R. R. Porter. 1980. Partial sequence of human complement component B: novel type of serine protease. *Proc. Natl. Acad. Sci. USA.* 77:4923.
20. Cooper, N. R., 1971. Enzymes of the complement system. *In* Progress in Immunology I. B. Amos, editor. Academic Press, New York. 567–577.
21. Lambris, J. D., and H. J. Muller-Eberhard. 1984. Isolation and characterization of a 33,000-dalton fragment of complement Factor B with catalytic and C3b binding activity. *J. Biol. Chem.* 259:12685.
22. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scan. J. Clin. Lab. Invest.* 21:S77.
23. Gmelig-Meyerling, F., and T. A. Waldmann. 1980. Separation of human blood monocytes and lymphocytes on a continuous percoll gradient. *J. Immunol. Methods.* 33:1.
24. Ly, I. A., and R. I. Mishell. 1974. Separation of mouse spleen cells by passage through columns of sephadex G-10. *J. Immunol. Methods.* 5:239.
25. Falkoff, R. M., M. Peters, and A. S. Fauci. 1982. T cell enrichment and depletion of human peripheral blood mononuclear cell preparations. *J. Immunol. Methods.* 50:33.
26. Muraguchi, A., J. L. Butler, J. H. Kehrl, and A. S. Fauci. 1983. Differential sensitivity of human B cell subsets to activation signals by anti-μ antibody and proliferation signals delivered by the monoclonal B cell growth factor. *J. Exp. Med.* 157:530.
27. Ambrus, J. L. Jr., and A. S. Fauci. 1987. Current studies examining regulation of the human B cell cycle. *In* Molecular Basis of Lymphokine Action. D. R. Webb, C. W. Pierce, and S. Cohen, editors. Humana Press Inc., Clifton, NJ. 137–148.
28. Lane, H. C., D. J. Volkman, G. Whalen, and A. S. Fauci. 1981. In vitro antigen-induced, antigen-specific antibody production in man. *J. Exp. Med.* 154:1043.
29. Ambrug, J. L., and A. S. Fauci. 1985. Human B lymphoma cell line producing B cell growth factor. *J. Clin. Invest.* 75:732.